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Effects of captopril and clonidine on resting and stress cardiac performance of left ventricle in spontaneously hypertensive rats[1]

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ABSTRACT Captopril (Cap) 20 $mg \cdot kg^{-1} \cdot d^{-1}$ and clonidine (Clo) 300 $\mu g \cdot kg^{-1} \cdot d^{-1}$ were given *po* to SHR from their parents mating day to 24 wk of age, with untreated, age-matched SHR and WKY as controls. Stress cardiac function was assessed by the development of $LVdp/dt_{max}$ in response to incremental pressure load (phenylephrine, Phe) and volume load (dextran, Dex). The slope of △HR/△MAP relationship curve was used as an index of baroceptor sensitivity. Results showed that both Cap and Clo caused decreases in BP in SHR. Cap not only reduced markedly the left ventricular mass/body weight (LVM/BW), ($mg \cdot g^{-1}$, 2.7 ± 0.4 *vs* SHR 3.5 ± 0.3, $P < 0.01$), but also normalized the $LVdp/dt_{max}$, $-LVdp/dt_{max}$, T value, and the stress cardiac function. Clo neither decreased the LVM/BW ($mg \cdot g^{-1}$, 3.4 ± 0.5 *vs* SHR 3.5 ± 0.3, $P > 0.05$), nor improved the resting and stress cardiac function. The results suggested that attenuation of the left ventricular hypertrophy (LVH) is beneficial not only to the resting but also stress cardiac performance.

KEY WORDS captopril; clonidine; heart function tests; phenylephrine; nitroprusside; pressoreceptors; inbred SHR rats; inbred WKY rats

Is the regression of the hypertensive left ventricular hypertrophy (LVH) beneficial? Both clinical and laboratory investigators are interested in this very practical and daily encountered issue[1-3]. Most of the antihypertensive drugs can reverse the LVH, but the regressive effect on the cardiac systolic and diastolic performance is variable[3-5]. Investigators speculated that reversal of hypertrophied myocytes is much easier than that of collagen hyperplasia, because of shorter half life of myocyte protein compared with that of interstitial fiber. They worried about that antihypertensive drugs would reverse the hypertrophied myocytes and leave relatively greater amount of collagen, leading to a stiffer ventricle. Furthermore, LVH is an adaptive response of the organism to a greater afterload imposed by hypertension, aiming at overcoming a greater resistance and maintaining a normal or even higher output[6,7]. They argued whether interruption of the adaptive process by various antihypertensive drugs may deteriorate the cardiac function. So far, investigators have been focusing their concerns on cardiac function after regression of LVH in established hypertension. However, no reports of early therapy to prevent the development of hypertension on resting and stress cardiac function have been found. This paper is intended to reveal whether captopril (Cap) treatment, initiate from their parents mating day, can improve the resting and stress cardiac function, the control group comprised of untreated WKY, untreated SHR, and clonidine (Clo)-treated SHR group.

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MATERIALS AND METHODS

Rats Experiments were performed on 24-wk-old spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats bred in our laboratory. The breeding rats were given Cap (20 mg·kg^{-1}·d^{-1}, Sino-American Shanghai Squibb Pharmaceutical, Ltd) and Clo (300 μg·kg^{-1}·d^{-1}, Danyang Pharmaceutical Factory, Jiangsu Province) *po* daily mixed in milk powder (about 10 g) as a cake, starting on the mating day throughout pregnancy and lactation. After weaning, the treatment of only the pups (n=13, 12 in Cap and Clo treated groups respectively) was maintained with the same dosage as that for their parents until 24 wk of age, when the experiment began. The control ♀ and ♂ breeders and their pups (untreated SHR n=13, untreated WKY n=13) were given only the vehicle. The rats were housed 3 to 4 per cage in a room at 22±2 ℃, humidity 55±5 %, and a 12-h light:dark cycle. Standard laboratory rat chow and tap water were provided *ad lib*.

Baroreflex control of heart rate The rats were anesthetized with sodium pentobarbital (35 mg·kg^{-1}, *ip*.) and one catheter was placed via the femoral artery into the descending aorta and another one through femoral vein into inferior vena cava for measuring the BP and administering the drugs. BP was monitored by using a solid transducer (model CYS, Institute 634 of Ministry of Astronautics, Beijing) and recorded on a polygraphy (model RM6200, Nihon Kohden Corp, Tokyo, Japan). The experiment commenced after BP and HR had stabilized. MAP and HR in response to bolus iv injection of Phe and nitroprusside (Nit) (0.5, 1, 2, 4, and 8 μg·kg^{-1}) were monitored. Drugs were dissolved in 0.9 % saline solution before each experiment every day, and the volume of injection was 0.2 ml. The sequence of administration of Phe and Nit as well as doses of various drugs was randomized. BP and HR were allowed to return to basal level before the next dose was given. Peak increase and decrease in MAP after Phe or Nit injection and the corresponding peak reflex changes in HR were recorded. HR was calculated by R-R period on the electrocardiogram: HR=3000/(R-R). The slope for baroreceptor control of HR was determined by fitting a regression line through points relating the changes in HR and the changes in MAP.

Measurement of left ventricular mass (LVM) Atria, the root of aorta, and the pulmonary artery were excised along the curve between atria and ventricles. After removal of the free wall of the right ventricle, the left ventricle (LV) with the septum was weighed as the LVM. LVM to BW ratio (LVM/BW) was used to quantitate the LVH.

Myocardial NE measurement Myocardium was homogenized in a glass grinder in ice cold perchloric acid 0.4 mmol·L^{-1} (50 ml·g^{-1} heart tissue, containing edetic acid 3 μmol·L^{-1} and sodium metabisulfate 10 μmol·L^{-1}). After centrifugalizing at 16 000×g at 4 ℃ for 20 min. NE in the supernatant was absorbed with activated albumin and measured by a spectrofluorometer. The sensitivity was 10 μg·g^{-1} myocardium with a recovery rate of 84±s 3 % and a coefficient of variation of 3.8 %.

Myocardial hydroxyproline determination The samples of LV were cleaned of blood and dried to constant weight at 40 ℃ and ground. The sample of tissue powder was heated with HCl 4 mmol·L^{-1} at 105 ℃ overnight for hydrolysis of collagen. Hydroxyproline content was measured spectrophotometrically after its reaction with Ehrlich reagent at 558 nm[10].

Measurement of cardiac pumping ability[9] Left ventricular and arterial pressure were obtained by catheters placed in the LV via the right carotid artery and femoral artery respectively and connected to solid state transducers (PT14M, Fudan University, Shanghai). These transducers were connected with a microcomputer detecting system (model WXG-I Railway Medical College, Nanjing), and a computer (Apple-II). Instantaneous changes in LV and arterial pressures were recorded and the results were calculated and printed by the computer. After catheterization and stabilization 30 min, the LVSP, LVEDP, t-dp/dt_{max}, LVdp/dt_{max}, −LVdp/dt_{max}, V_{max}, and the time of the pressure decrease in the period of isovolumic relaxation (T value) were recorded. Then escalating doses of 6 % dextran (Dex) 1, 2, 4, and 8 ml·kg^{-1} and Phe 0.5, 1, 2, and 4 μg·kg^{-1} of Phe were administrated. The responses of LVdp/dt_{max} to the increasing volume and pressure load were regarded as the stress cardiac performance.

Statistical analysis ANOVA and unpaired t test were used to evaluate any difference between the control and treated animals.

RESULTS

At 24 wk of age, SBP, DBP, and MAP in untreated SHR were higher than those in WKY ($P<0.01$), with no difference in HR and BW, and LVM/BW in untreated SHR was much greater than that in WKY ($P<0.01$). Both Cap and Clo treatments early started effectively prevented the increases in BP in SHR. Although SBP reduction by Cap did not reach the level of that of WKY, MAP decreased as much as that of WKY. Meanwhile, LVM/BW was decreased by Cap (SHR_{Cap} 2.7 ± 0.4 *vs* SHR 3.5 ± 0.3, $P<0.01$). Although Clo decreased SBP, DBP, and MAP to an extent of WKY, while no significant effect on LVM/BW has been shown (SHR_{Clo} 3.4 ± 0.5 *vs* SHR 3.5 ± 0.3, $P>0.05$) (Tab 1).

Myocardial NE and hydroxyproline Myocardial NE and hydroxyproline in untreated SHR were increased. Both Cap and Clo treatments decreased the myocardial NE and hydroxyproline in SHR. The decrease in hydroxyproline in Cap treated rats was more than that in Clo treated group (Tab 1).

Cardiac pumping ability At 24 wk of age, in the resting state, $LVdp/dt_{max}$, and V_{max} were greater in SHR than that in WKY ($LVdp/dt_{max}$ SHR 2155±390 *vs* WKY 1455±456 kPa·s^{-1}, $P<0.01$), with less $-LVdp/dt_{max}$ and higher T value (Tab 1), indicating an enhancement of cardiac contractility and impairment of diastolic function in untreated SHR. Cap treatment increased the $-LVdp/dt_{max}$ (SHR_{Cap} 1293±120 *vs* SHR 809±223 kPa·s^{-1}, $P<0.01$), with much lower T value ($P<0.01$). Meanwhile, LVEDP decreased by Cap compared with that in untreated SHR group, indicating that Cap improved the diastolic performance of SHR in resting state, and the abnormally accentuated hemodynamic systolic function was also ameliorated.

On the contrary, Clo increased the LVEDP and decreased the $LVdp/dt_{max}$, with

Tab 1. Effects of early start in rats from their parents mating day to 24-wk-old *po* captopril 20 mg·kg^{-1}·d^{-1} and clonidine 300 μg·kg^{-1}·d^{-1} treatment on BP, HR, body weight, LVM/BW, NE, hydroxyproline and heart function. $^{c}P<0.01$ *vs* SHR_{CTRL}, $^{f}P<0.01$ *vs* WKY. $\bar{x}\pm s$.

	WKY	SHR_{CTRL}	SHR_{Cap}	SHR_{Clo}
n	13	13	13	12
Body weight, g	322±63	289±31	267±52	248±45^{cf}
SBP, kPa	18.3±2.9	29.0±3.9^{f}	21.1±1.7^{c}	19.0±3.3^{c}
DBP, kPa	13.5±2.3	20.6±3.2^{f}	14.2±1.2^{c}	13.2±3.5^{c}
MAP, kPa	15.6±2.5	23.1±3.5^{f}	16.5±1.3^{c}	15.3±3.1^{c}
HR, bpm	352±46	355±32	363±31	255±36^{c}
LVM/BW, mg·g^{-1}	2.3±0.2	3.5±0.3^{f}	2.7±0.3^{c}	3.4±0.5^{f}
NE content, ng	307±124	1 236±225^{f}	458±231^{c}	554±121^{c}
Hydroxyproline, ng	533±143^{c}	766±116	529±135^{c}	603±115^{c}
LVSP, kPa	17±3	27±3^{f}	19±2^{f}	18±3^{c}
LVEDP, kPa	0.7±0.4	0.7±0.4	0.5±0.2	1.1±0.4^{c}
$LVdp/dt$, kPa·s^{-1}	1 454±456	2 155±390^{f}	1 602±239^{c}	1 127±217^{c}
t-dp/dt, ms	13.6±2.0	14.0±1.6	13.9±1.0	13.7±2.6
$-LVdp/dt$, kPa·s^{-1}	1 042±268	809±223	1 293±120^{c}	953±229
T, ms	11.9±2.6	15.2±3.0^{f}	11.6±2.6^{c}	16.3±3.9^{f}
V_{max}, s^{-1}	276±55	300±54	273±40	242±49

no difference in $-LVdp/dt_{max}$ and T values *vs* the untreated SHR, suggesting that Clo deteriorated the resting cardiac systolic and diastolic performance.

The change in $LVdp/dt_{max}$ related to the increment of pressure load by iv Phe was decreased in untreated SHR compared with that of WKY, especially in response to higher doses of Phe, suggesting that the potential impairment of cardiac systolic performance in the presence of high afterload, even though the resting cardiac systolic function was apparently enhanced. In Cap treated rats, however, the change in $LVdp/dt_{max}$ was much more increased than that of SHR. Clo treatment

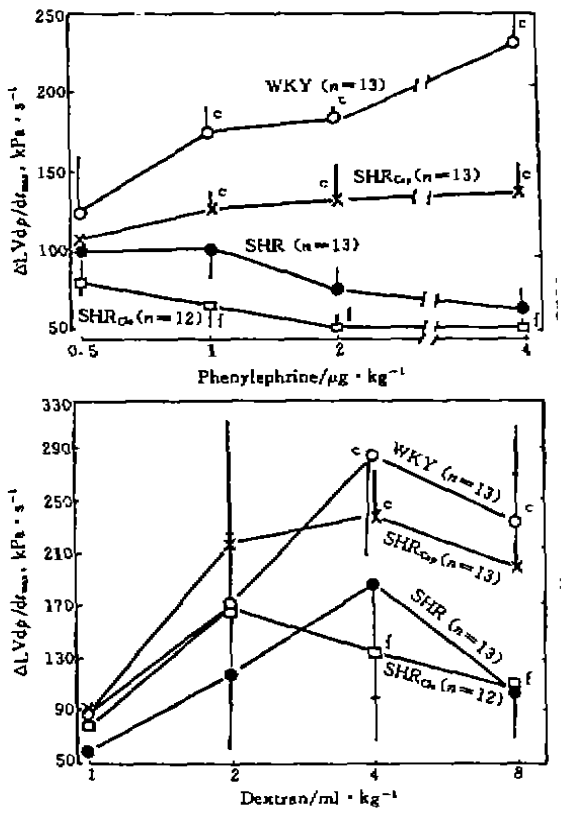

Fig 1. $LVdp/dt_{max}$ induced by iv phenylephrine or dextran in SHR rats treated with captopril (SHR_{Cap}) and clonidine (SHR_{Clo}) *vs* untreated SHR and WKY rats. $\bar{x}\pm s$. $^cP<0.01$ *vs* SHR, $^fP<0.01$ *vs* WKY.

deteriorated the systolic ability of LV compared with that of untreated SHR (Fig 1). With increasing volume load by iv Dex, the Frank-Starling curve rose initially in WKY rats, but declined when the dose of Dex reached 8 $ml\cdot kg^{-1}$. However, the Frank-Starling curve in the untreated SHR shifted rightward (Fig 1), suggesting that the systolic ability of LV was impaired also in the presence of over volume load in untreated SHR. Cap improved the volume stress cardiac systolic function, manifested by the F-S curve lying between those of SHR and WKY, while the curve was similar to that of untreated SHR in Clo group.

Baroreflex control of HR The sensitivity of baroreflex in the Cap treated SHR increased more markedly than that in untreated SHR. However, in the Clo treated SHR, the response of HR to change of BP was much attenuated than that in untreated SHR (Tab 2).

Tab 2. △HR/△MAP ($bpm\cdot kPa^{-1}$) of early start in rats from their parents mating day to 24-wk-old *po* captopril 20 $mg\cdot kg^{-1}\cdot d^{-1}$ and clonidine 300 $\mu g\cdot kg^{-1}\cdot d^{-1}$ treatment on the response in HR to the change in blood pressure. $^cP<0.01$ *vs* SHR_{CTRL}, $^fP<0.01$ *vs* WKY. $\bar{x}\pm s$.

	WKY ($n=13$)	SHR_{CTRL} ($n=13$)	SHR_{Cap} ($n=13$)	SHR_{Clo} ($n=12$)
Phe	5.3 ± 1.8^c	3.7 ± 1.1	5.8 ± 2.3^c	1.7 ± 0.9^{cf}
Nit	4.7 ± 2.5^c	3.5 ± 1.4	5.4 ± 1.7^c	1.7 ± 0.8^{cf}

DISCUSSION

In the present study, the rats received Cap without water but with a cake of milk powder in fasting state when the rats rushed to snatch at it. The advantage of this administration route compared with that with water drinking was that the dosage of the given drugs would be more accurate, less wasteful and completely under control, although longer

breeding time was needed.

The main findings of the present study were early treatment with either Cap or Clo could prevent the development of hypertension in SHR. This finding in Cap treatment was consistant with Cheng *et al*[11], but Clo early treatment to prevent the development of hypertension in SHR was not found now yet.

The results of previous studies on the LV performace after the regression of LVH by antihypertensive therapy were controversial. The effect of early treatment in SHR on the cardiac function was not reported. Pfeffere[5] found that the regression of LVH could prevent the deterioration of systolic performance of LV in aged SHR treated with guanethidine. Ferrario[12] also arrived at the same conclusion in renal hypertensive rats and SHR treated with methyldopa. Through clinical study, Fourad[13] indicated that the filling rate of LV was quicked significantly in patients with hypertension treated with metoprolol. White's[8] finding was similar to that of Fourad. But Inouye[14] could not find the improvement of LV performance in the hypertensive patient treated with a combination of diauretics and β receptor blocker or calcium antagonist. Similarly, Shali[7] failed to ascertain the improvement of the LV performance detected by ultrasonic Doppler technique after regression of LVH.

This study proved that Cap treatment not only prevented the occurrence of LVH, but improved the resting systolic and diastolic performance of LV. Moreover, there were evidences that when the volume and pressure load were imposed, the LV systolic performance were also improved by Cap compared with that of untreated SHR. Our study may be meaningful in solving the problem that has long puzzled clinicians, whether or not regression of LVH is beneficial to the cardiac performance.

Both Cap and Clo treatments reduced the hydroxyproline in myocardium, but the former inhibited the development of LVH and improved the resting and stress cardiac performance, while the latter was unable to prevent the development of LVH, even further deteriorated the resting and stress cardiac performance. Thus it is suggested that the amount of interstitial fiber may not be related to the cardiac performance as postulated by many investigators, but the extent of hypertrophy was actually in close relation to the cardiac performance.

Although many studies indicated that the prevention and regression of hypertensive development may restore the sensitivity of baroreflex[11,15], the exact mechanism has not yet been explored. Previous studies of the baroreceptor sensitivity suggested that, the changes in the sensitivity were mainly related to baroreceptors themselves, to the activity of the cardiovascular center and to the level of the arterial BP. Little attention has been paid to the relationship between the baroreceptor sensitivity and LVH. Our study found that the sensitivity of reflex controlling HR was enhanced significantly in Cap treated group *vs* untreated SHR, while the sensitivity of the arterial baroreceptor was reduced by Clo, even though BP in Clo treated group was comparable to that of Cap group, suggesting that the more obvious the LVH, the more inert the reaction of baroceptor response to the elevation of BP will be. The LVH may play an important role in the resetting of the arterial baroreceptor.

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卡托普利和可乐定对自发性高血压大鼠左心室静息及负荷功能的影响

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A **摘要** 卡托普利(Cap)和可乐定(Clo)极早期治疗SHR大鼠，观察BP，LVM/BW，压力反射敏感性，静息心脏功能及前后负荷增加时$\triangle LVdp/dt_{max}$的变化。Cap，Clo均明显防止SHR大鼠BP的升高。Cap不仅降低LVM/BW，且明显改善静息及前后负荷增加时左室收缩与舒张功能。同时Cap明显增加压力反射敏感性。而Clo则无此作用。且可能左室肥厚与压力反射敏感性有关。

关键词 卡托普利；可乐定；心功能试验；压力感受器；苯福林；硝普钠；近交SHR大鼠；近交WKY大鼠